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Art. VIII.—*A Staining and Maceration Method of Tracing the Path of the Vascular Bundles in Herbaceous Plants, and its Application in Observations on the Distribution of* Bacterium solanacearum *in Relation to Epinastic Curvatures in Petioles of Tomato and Potato Plants.*

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Introduction.

In certain plants infected by *Bacterium solanacearum* it has been shown (3, 5, 6) that petiole-epinasty and adventitious root production occur. In experiments designed to show the relation of the bacteria to the epinasty and root formation, the following method was developed.

Healthy tomato plants were first used. The stems were severed at the base, and the plants placed in 1 per cent. solutions of eosin or basic fuchsin from one to two hours, until the dye was definitely visible in the veins of the topmost leaves. After removal from the dye solution, the petioles were cut off at a distance of about one-half inch from the stem. The epidermis was then slit along the stems and petiole bases with a sharp scalpel, and the plants immersed in dilute nitric acid (15 per cent.) which had been raised to boiling point. This strength of acid had been found suitable by Wilson (7) in his experiments to obtain epidermal tissues from leaves. The period of immersion in the acid was a few seconds in the case of tomato stems, but varied from one to five minutes for well developed potato stems. The plant was generally found to be in a suitable state of limpness for dissection by the time numerous bubbles were seen arising from the cut ends of the stem immersed in the gently boiling acid. On removal from the acid the plants were washed in running water for ten minutes, and then allowed to stand in water overnight. The epidermal and cortical tissues could then be easily dissected away with fine pointed forceps. The vascular tissue and the eosin and basic fuchsin dyes were unaffected by the acid treatment so that the path of the bundles could be easily traced. Other dyes, particularly light green as recommended by Harvey (4) were tried, but this dye and certain of the others showed a tendency after the acid treatment to lose colour or to become diffused throughout the parenchyma tissue. The red dyes also afforded a greater visual and photographic colour contrast with the bacterially filled bundles.

The method is specially useful in anatomical work on herbaceous plants and obviates in large measure the laborious serial sectioning method of recording the path and forkings of the bundles. Thus for potato, it enables one to trace the course of the bundles and to confirm Artschwager's (1) reconstruction of them made from a great number of transverse sections. The method was next applied to tomato and potato plants in various stages of the disease caused by the vascular parasite *Bacterium solanacearum*. The dye was taken up either through the base of the cut stem or through cut roots. Vessels clogged with bacteria failed to allow the passage of the stain, and on dissection, the infected bundles showed a yellowish appearance in contrast to the red of the non-bacterially invaded vessels. The method incidentally provides an illustration of the fact that the water of the transpiration stream travels essentially in the lumen of the vessels. Where the invasion and clogging of the vessels was general, the stain did not penetrate upwards at all.

In order to outline more clearly the longitudinal extent of the bacterially-invaded parts of the vessels, advantage was taken of the fact that on cutting a petiole or leaf near the top of the plant under a water solution of the dye, some of this is sucked back into the vascular system of the plant by the negative pressure in the water system. Using the method of Caldwell (2), and a modification of it, whereby plasticene cups to hold the dye were built around the petiole, it was possible to delimit fairly accurately the infected vessels from above and then from below, by the uptake from the roots. As a check on the technique, transverse sections of the petioles were cut about one-half inch out from the stem before its treatment with acid. The presence or absence of bacteria could be determined in this way, and then checked by the maceration method. The staining method facilitated the determination of the presence or absence of bacteria in the vessels in the examination of transverse sections.

In connexion with the adventitious root formation which occurs in tomato as a reaction to infection by *B. solanacearum*, it was of interest to trace the line of development of the roots along the stem, and to determine where they developed in relation to the bacteria. The stain and maceration method proved quite useful here provided that more care was exercised in dissecting away the tissue around the developing roots.

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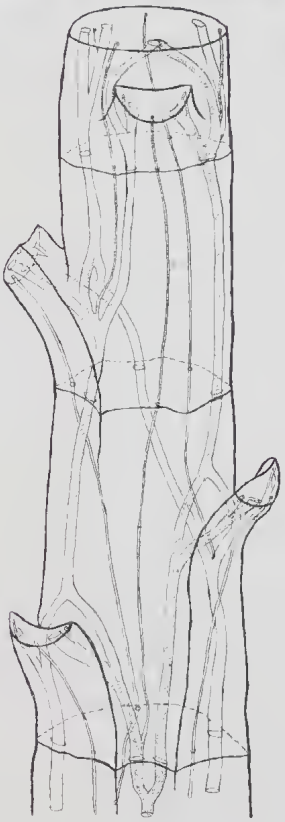
Explanation of Plate IV.

Fig. 1.—Diagrammatic representation of the course of the vascular bundles in *Solanum tuberosum* as obtained by the staining and maceration method. The path taken by the invading bacteria is indicated by the dots.

Fig. 2.—Potato stem dissected to show the path of the vascular bundles. All bundles are full of bacteria and appear yellow in the preparation.

Fig. 3.—Potato stem dissected. The dark appearing bundles are free of bacteria and have taken up the eosin, while the light coloured bundles are full of bacteria.



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